Journal of Fisheries
Volume 4 Issue 3 Pages: 428-430 December 2016
Peer Reviewed | Open Access | Online First

eISSN 2311-3111
pISSN 2311-729X

Short Communication
DOI: dx.doi.org/10.17017/jfish.v4i3.2016.158

Transferable chloramphenicol resistance determinant in luminous *Vibrio harveyi* from penaeid shrimp *Penaeus monodon* larvae

Thangapalam Jawahar Abraham

Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Kolkata 700 094, India

Correspondence
Dr TJ Abraham; Department of Aquatic Animal Health, West Bengal University of Animal and Fishery Sciences
Email: abrahamtj1@gmail.com

Manuscript history
Received: 27 Apr 2016; Received in revised form: 14 Nov 2016; Accepted: 25 Nov 2016; Published online: 31 Dec 2016

Citation
Abraham TJ (2016) Transferable chloramphenicol resistance determinant in luminous *Vibrio harveyi* from penaeid shrimp *Penaeus monodon* larvae. Journal of Fisheries 4(3): 428-430. DOI: 10.17017/jfish.v4i3.2016.158

Abstract

Antibiotic-resistant luminous *Vibrio harveyi* strains isolated from *Penaeus monodon* larvae were screened for the possession of transferable resistance determinants. All the strains were resistant to chloramphenicol and the determinant coding for chloramphenicol resistance was transferred to *Escherichia coli* at frequencies of $9.50x10^{-4}$ to $4.20x10^{-4}$. The results probably suggest the excessive use of chloramphenicol in shrimp hatcheries to combat luminous vibriosis.

Keywords: *Penaeus monodon*; drug resistance; *Vibrio harveyi*; resistance determinants

INTRODUCTION

A number of antibacterial compounds have been used in aquaculture during various stages of production. Bacterial resistance to antibiotics is a widely recognized phenomenon and in many species of bacteria, antibiotic-resistance is associated with plasmids particularly for antibacterials such as cephalosporin, chloramphenicol, penicillin, tetracyclines, trimethoprim and sulfonamides. Bacteria develop resistance to these antibiotics by decreasing the cell membrane permeability. In contrast, chromosomal mutation is the mechanism responsible for the bacterial resistance to quinolones (Hernandez 2005). R-plasmids were detected in various fish pathogens such as *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Pasteurella piscicida*, *Vibrio* sp., *Streptococcus* sp. (Aoki 1992), *E. ictaluri* (Starliper *et al.* 1993), *V. anguillarum* (Nakajima *et al.* 1983) and *V. harveyi* (Abraham 2006). The luminous bacteria are recognised as tropical pathogens of economic importance especially as the causative agent of luminous vibriosis in penaeid shrimp larvae. To combat luminous vibriosis a wide variety of antibiotics are used in hatcheries. Earlier studies revealed high proportion of antibiotic resistant bacteria in Indian shrimp farming systems (Abraham *et al.* 1997; Abraham 2006; Vaseeharan *et al.* 2008). The present communication reports the results of the experiments conducted to determine whether or not the antibiotic-resistant luminous bacteria from shrimp larvae possess transferable resistance determinants.

METHODOLOGY

A total of five antibiotic-resistant luminous *Vibrio harveyi* strains (S_1-S_5) isolated from hatchery raised shrimp larvae (*Penaeus monodon*), as described in Abraham *et al.* (1997), were used as donor strains for the conjugal plasmid transfer assay based on their resistance phenotype and minimal inhibitory concentration (MIC) of chloramphenicol. Recipient strain, *Escherichia coli* BP_3

was isolated from a brackishwater penaeid shrimp pond in Malancha, 24 Parganas District, West Bengal, India. Conjugative plasmid transfer assays were carried out by mating in Luria-Bertani (LB) medium as described by Provence and Curtiss (1994) with slight modification. In brief, 50% of tryptone in LB medium was replaced with peptone to facilitate the growth of marine luminous bacteria. Loopful of young cells of antibiotic-resistant donor and antibiotic sensitive recipient strains from agar slants were inoculated separately into 5 ml each of LB medium contained in test tubes and incubated overnight without agitation at 30 °C. These overnight grown parent cultures were diluted by 1:20 with fresh LB medium, allowed to grow for another 4 h and the cell densities were adjusted to $5.00x10^7$–$2.00x10^8$/ml. Nine ml each of recipient culture was transferred to a series of sterile flat-bottomed mating flasks of 100 ml capacity. The number of recipient cells in LB medium was determined on spread plates with bromothymol blue lactose nutrient (BLN) agar (Zhao *et al.* 1992) containing 0.35% sodium chloride (NaCl). After 5 min, one ml each of donor cultures were introduced into the respective mating flasks and mixed gently. The number of donor cells in LB medium was determined on spread plates with seawater complex agar. The mating flasks were incubated at 30 °C for 3 h without agitation. Cultures (0.1 ml each) were then spread onto BLN agar containing chloramphenicol (25 µg/ml) for the selection of transconjugants. Colonies that appeared yellow on chloramphenicol-BLN agar were considered transconjugants and purified twice on a similar medium. Transconjugants were confirmed as *E. coli* clones based on their ability to utilize lactose and produce gas from glucose. *Vibrio harveyi* is negative for lactose utilization and gas production from glucose (Holt *et al.* 1994). Buffered saline was used as diluent.

The frequency of transfer and transfer efficiency were expressed in terms of the number of transconjugants per number of donor strain cells and the number of transconjugants per number of recipient cells, respectively. Mutation frequency of recipient E. coli strain was determined by spreading 0.1 ml each of 10^0-10^{-3} dilutions onto BLN agar containing 25 µg/ml of chloramphenicol. The antibiogram and determination of minimal inhibitory concentration (MIC) of chloramphenicol for the donors, recipient, mutant and transconjucants were done as described in Abraham (2006). The antibiotics (µg/disc) included in the antibiogram assay were chloramphenicol (30), ciprofloxacin (5), erythromycin (15), furazolidone (50), gentamycin (10), nalidixic acid (30), nitrofurantoin (300), novobiocin (30), oxytetracycline (30), sulfamethizole (300), tetracycline (300) and trimethoprim (5).

RESULTS AND DISCUSSION

All the donor strains of *V. harveyi* (S_1-S_5) from shrimp larvae were resistant to chloramphenicol, showing MIC values in the range of 75-100 µg/ml. These results suggested that numerous aquatic bacteria that are resistant to chloramphenicol may be present in the system and may have public health significance. The recipient *E. coli* BP_3 was sensitive to all the antibiotics tested and the MIC of chloramphenicol was 3.13 µg/ml. Plasmids that are capable of crossing the borders between bacterial families are normally defined as broad host range plasmids (Sandaa and Enger 1996). Such a broad host range resistance determinant was present in all *V. harveyi* strains of this study. The determinant coding for resistance to chloramphenicol was transferred to *E. coli*, a member of Enterobacteriaceae, at frequencies of $9.50x10^{-4}$–$4.20x10^{-4}$ (Table 1), a finding which might well be explained by the abundant use of antibiotics in hatcheries. The determinant's transfer efficiency was ranged between $7.40x10^{-4}$ and $1.50x10^{-4}$ (Table 1). The rate at which *E. coli* mutated to develop resistance to chloramphenicol at 25 µg/ml was determined to confirm whether the cells were conjugating or mutating. The observed mutation frequency was in the range of $4.20x10^{-9}$–$6.00x10^{-8}$, which confirmed that the cells grown on chloramphenicol-BLN agar after mating were transconjugants and not mutants. Using *E. coli* as a recipient, Toranzo *et al.* (1984) noted transfer of plasmids in the range of 10^{-4}–10^{-7} in bacteria isolated from cultured rainbow trout. Similar results were obtained in other experiments where *E. coli* was used as a recipient for a plasmid isolated from the natural environment (Aoki 1992) and shrimp larvae (Abraham 2006). The observations of the present study indicated that the plasmid of luminous bacteria coding for resistance to chloramphenicol has a moderately high frequency of transfer. Similarly, Sandaa and Enger (1996) recorded possession of a broad host range plasmid in *A. salmonicida* with a high frequency of transfer to *E. coli*.

Table 1: Transfer frequencies of chloramphenicol-resistance determinant of luminous *Vibrio harveyi* to recipient *Escherichia coli* BP_3

Donor strains	*Frequency of transfer ($\times 10^{-4}$)*	*Transfer efficiency ($\times 10^{-4}$)*
Vibrio harveyi S_1	5.70	7.40
V. harveyi S_2	4.20	5.10
V. harveyi S_3	4.40	3.70
V. harveyi S_4	9.50	3.90
V. harveyi S_5	2.40	1.50

Mutation frequency of recipient *E. coli* BP_3 to chloramphenicol (25 µg/ml) was in the range of $4.20 \times 10^{-9} - 6.00 \times 10^{-8}$.

Besides chloramphenicol, the determinants coding for sulfamethizole and trimethoprim resistance were also transferred from *V. harveyi* to *E. coli*. The determinant of intermediate level resistance to sulphamethizole was, however, not transferred to *E. coli* so also in a study on *Streptococcus* sp. by Aoki *et al.* (1990). The transfer of furazolidone and nitrofurantoin resistance determinants from luminous vibrios to the recipient *E. coli* was not observed, which corroborate the observations of Zhao *et al.* (1992). It is well known that surfaces with high bacterial densities are sites where plasmid transfer is most likely to occur. Transfer of plasmids between the strains of *E. coli* under rumen conditions was also reported (Scott and Flint 1995). It is, therefore, likely that shrimp intestines, nutrient rich hatchery rearing water and aquaculture pond sediments may provide ideal conditions for bacterial proliferation and that would favour antibiotic resistance determinant's transfer. In recent years, special attention has been given to the possibility of transfer of resistance genes from bacteria in the environment to bacteria pathogenic to fish, shrimp and human beings. In fact, earlier studies demonstrated conjugal R-plasmid transfer from *V. anguillarum* to *E. coli*, from *V. anguillarum* to *V. cholerae* (Nakajima *et al.* 1983; Sandaa *et al.* 1992) and from *A. salmonicida* to luminous *V. fischeri* and other non-luminous bacteria (Sandaa and Enger 1996). Thus, it is possible that the bacterial flora of shrimp larvae may provide a reservoir of antibiotic resistance determinants transmissible to other aquatic bacterial flora, which share that same aquatic environment. This situation highlights the need for the responsible use of antibiotics in aquaculture as described in Hernandez (2005).

ACKNOWLEDGEMENTS

The research work was financed by the Indian Council of Agricultural Research, Government of India, New Delhi.

REFERENCES

Abraham TJ, Manley R Palaniappan R and Dhevendaran K (1997) Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimps. Journal of Aquaculture in the Tropics 12: 1-8.

Abraham TJ (2006) Virulence of *Vibrio harveyi* possessing a transferable chloramphenicol resistance determinant to larvae of Indian white shrimp *Fenneropenaeus indicus* (Decapoda). Indian Journal of Marine Science 35(3): 275–278.

Aoki T (1992) Chemotherapy and drug resistance in fish farms in Japan. In: Shariff M, Subasinghe RP and Arthur JR (Eds.), Diseases in Asian Aquaculture, Vol. 1, Fish Health Section, Asian Fisheries Society, Manila, pp. 519-529.

Aoki T, Takami K and Kitao T (1990) Drug resistance in a non-haemolytic *Streptococcus* sp. isolated from cultured yellow tail *Seriola quinqueradiata*. Diseases of Aquatic Organisms 8: 171-177.

Hernandez SP (2005) Responsible use of antimicrobials in aquaculture. FAO Fisheries Technical Paper No. 469, FAO, Rome, Italy. 97 pp.

Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST (1994) Bergey's manual of determinative bacteriology. 9th edition. Williams and Wilkins, Baltimore. pp. 260-274.

Nakajima T, Suzuki M, Harada K, Inoue M and Mitsuhashi S (1983) Transmission of R-plasmids in *Vibrio anguillarum* to *Vibrio cholerae*. Microbiology and Immunology 27: 195-198.

Provence DL and Curtiss III R (1994) Gene transfer in gram negative bacteria. In: Gerhardt P, Murray RGE, Wood WA and Krieg NR (Eds.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington DC. pp: 317-347.

Sandaa RA and Enger O (1996) High frequency transfer of a broad host range plasmid present in an atypical strain of the fish pathogen *Aeromonas salmonicida*. Diseases of Aquatic Organisms 24: 71-75.

Sandaa RA, Torsvik VL and Goksoyr J (1992). Transferable drug resistance in bacteria from fish farm sediments. Canadian Journal of Microbiology 38: 1061-1065.

Scott KP and Flint HJ (1995) Transfer of plasmids between strains of *Escherichia coli* under rumen conditions. Journal of Applied Bacteriology 78: 189-193.

Starliper CE, Cooper RK, Shotts EB Jr, Tayler PW (1993) Plasmid-mediated Romet resistance of *Edwardsiella ictaluri*. Journal of Aquatic Animal Health 5: 1-8.

Toranzo AE, Combarro P, Lemos ML and Barja JL (1984). Plasmid coding for transferable drug resistance in bacteria isolated from cultured rainbow trout. Applied Environmental Microbiology 48: 872-877

Vaseeharan B, Hussian MR and Cheng JC (2008) RpoN gene, RAPD profile, antimicrobial resistance and plasmids of *Vibrio anguillarum* isolates from vibriosis infected *Penaeus monodon*. Letters in Applied Microbiology 47(5): 380-385.

Zhao J, Kim E, Kobayashi T and Aoki T (1992) Drug resistance of *Vibrio anguillarum* isolated from Ayu between 1989 and 1991. Nippon Suisan Gakkashi 58: 1523-1527.